

Attorney Docket No.: RU-0064
Inventors: Lazarus et al.
Serial No.: 09/332,886
Filing Date: June 15, 1999
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This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

Claim 1 (original): A mutant form of 2,5-DKG reductase A having improved ability to convert 2,5-DKG into 2-KLG.

Claim 2 (original): The mutant according to claim 1 having an amino acid substitution in a position of 2,5-DKG reductase A selected from the group consisting of 165, 166, 167, 168, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, and 278.

Claim 3 (original): The mutant according to claim 1 having an amino acid substitution in position 192.

Claim 4 (original): The mutant according to claim 3 having an arginine at position 192.

Claim 5 (original): A DNA construct comprising a structural gene containing at least one mutated codon, said gene coding for a mutant form of 2,5-DKG reductase A having improved ability to convert 2,5-DKG into 2-KLG.

Claim 6 (original): A DNA construct according to claim 5, wherein said mutated codon is a codon selected from the group

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consisting of 165, 166, 167, 168, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, and 278 which results in an amino acid substitution in a position of 2,5-DKG reductase A selected from the group consisting of 165, 166, 167, 168, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, and 278.

Claim 7 (original): A DNA construct according to claim 5, wherein said mutated codon is codon 192 which results in an amino acid substitution in position 192 of 2,5-DKG reductase A.

Claim 8 (original): A DNA construct according to claim 7 which results in arginine at position 192.

Claim 9 (original): A mutant form of 2,5-DKG reductase A having increased expression.

Claim 10 (original): The mutant according to claim 9 having amino acid substitutions in positions 2, 5, and 7 of 2,5-DKG reductase A.

Claim 11 (original): The mutant according to claim 10 having an asparagine at position 2, a threonine at position 5, and a serine at position 7.

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Claim 12 (original): A DNA construct comprising a structural gene containing at least one nucleotide substitution, said gene coding for a 2,5-DKG reductase A resulting in increased expression of said 2,5-DKG reductase A.

Claim 13 (original): A DNA construct according to claim 12, wherein said nucleotide substitutions are in codons 2, 5, and 7 which result in amino acid substitutions in positions 2, 5, and 7 of 2,5-DKG reductase A.

Claim 14 (original): A DNA construct according to claim 13 which results in asparagine at position 2, threonine at position 5, and serine at position 7.

Claim 15 (original): A host cell transformed with an expression vector that includes a DNA construct according to claims 5, 6, 7, 8, 12, 13 or 14.

Claim 16 (original): The host cell of claim 15 which is a bacterium.

Claim 17 (original): The host cell of claim 16, wherein the bacterium is of the genus *Erwinia*.

Claim 18 (original): The host cell of claim 16, wherein the bacterium is of the genus *Gluconobacter*.

Claim 19 (original): The host cell of claim 16, wherein the bacterium is of the genus *Acetobacter*.

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Claim 20 (original): The host cell of claim 19, wherein the bacterium is *Acetobacter cerinus* (IFO 3263).

Claim 21 (original): The host cell of claim 14, wherein the expression vector is a plasmid.

Claim 22 (original): The host cell of claim 21, wherein the plasmid is pSstac.DKGR.AAA.HS1.

Claim 23 (original): A mutant form of 2,5-DKG reductase A having improved temperature stability.

Claim 24 (original): The mutant according to claim 23 having amino acid substitutions in positions 55 and 57 of 2,5-DKG reductase A.

Claim 25 (original): The mutant according to claim 24 having an alanine at positions 55 and 57.

Claim 26 (original): A DNA construct comprising a structural gene containing at least one mutated codon, said gene coding for a mutant form of 2,5-DKG reductase A having improved temperature stability.

Claim 27 (original): A DNA construct according to claim 26, wherein said mutated codons are codons 55 and 57 which result in amino acid substitutions in positions 55 and 57 of 2,5-DKG reductase A.

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Claim 28 (original): A DNA construct according to claim 27 which results in alanine at positions 55 and 57.

Claim 29 (original): A mutant form of 2,5-DKG reductase A having improved ability to convert 2,5-DKG into 2-KLG, having increased expression, and having improved temperature stability.

Claim 30 (original): The mutant according to claim 29 having amino acid substitutions in positions 192, 2, 5, 7, 55, and 57 of 2,5-DKG reductase A.

Claim 31 (original): The mutant according to claim 30 having an arginine at position 192, an asparagine at position 2, a threonine at position 5, a serine at position 7, and an alanine at positions 55 and 57.

Claim 32 (original): A DNA construct comprising a structural gene containing at least six mutated codons, said gene coding for a mutant form of 2,5-DKG reductase A having improved ability to convert 2,5-DKG into 2-KLG, having increased expression, and having improved temperature stability.

Claim 33 (original): A DNA construct according to claim 32, wherein said codons are codons 192, 2, 5, 7, 55, and 57 which result in amino acid substitutions in positions 192, 2, 5, 7, 55, and 57 of 2,5-DKG reductase A.

Claim 34 (original): A DNA construct according to claim 33 which results in arginine at position 192, asparagine at position

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2, threonine at position 5, serine at position 7, and alanine at positions 55 and 57.

Claim 35 (original): A host cell transformed with an expression vector that includes a DNA construct according to claims 32, 33 or 34.

Claim 36 (original): The host cell of claim 35 which is a bacterium.

Claim 37 (original): The host cell of claim 36, wherein the bacterium is of the genus *Erwinia*.

Claim 38 (original): The host cell of claim 36, wherein the bacterium is of the genus *Gluconobacter*.

Claim 39 (original): The host cell of claim 36, wherein the bacterium is of the genus *Acetobacter*.

Claim 40 (original): The host cell of claim 39, wherein the bacterium is *Acetobacter cerinus* (IFO 3263).

Claim 41 (original): The host cell of claim 35, wherein the expression vector is a plasmid.